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Evaluation of coronary stenosis with the aid of quantitative image analysis in histological cross sections

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ABSTRACT

Coronary artery atherosclerosis is a hugely prevalent condition in the Western World and is often encountered during autopsy. Atherosclerotic plaques can cause luminal stenosis: which, if over a significant level (75%), is said to contribute to cause of death. Estimation of stenosis can be macroscopically performed by the forensic pathologists at the time of autopsy or by microscopic examination. This study compares macroscopic estimation with quantitative microscopic image analysis with a particular focus on the assessment of significant stenosis (>75%). A total of 131 individuals were analysed. The sample consists of an atherosclerotic group ($n = 122$) and a control group ($n = 9$). The results of the two methods were significantly different from each other ($p = 0.001$) and the macroscopic method gave a greater percentage stenosis by an average of 3.5%. Also, histological examination of coronary artery stenosis yielded a difference in significant stenosis in 11.5% of cases. The differences were attributed to either histological quantitative image analysis underestimation; gross examination overestimation; or, a combination of both. The underestimation may have come from tissue shrinkage during tissue processing for histological specimen. The overestimation from the macroscopic assessment can be attributed to the lumen shape, to the examiner observer error or to a possible bias to diagnose coronary disease when no other cause of death is apparent. The results indicate that the macroscopic estimation is open to more biases and that histological quantitative image analysis only gives a precise assessment of stenosis *ex vivo*. Once tissue shrinkage, if any, is accounted for then histological quantitative image analysis will yield a more accurate assessment of *in vivo* stenosis. It may then be considered a complementary tool for the examination of coronary stenosis.

1. Introduction

In order for significant atherosclerosis (coronary artery disease) to be considered a contributing factor to the death of an individual, at least one of the major coronary arteries must have more than 75% luminal stenosis.[1] Reliable and accurate assessment of stenosis is essential for establishing cause of death and in some cases manner of death.[2–5] Exact percentages are especially relevant in cases where there is moderate stenosis with questions arising on life expectancy. There are two methods used to determine the percentage of stenosis. One is a gross visual assessment performed by the pathologist during the autopsy and the other requires the use of histological methods.

Gross visual examination of coronary arteries is simple and inexpensive but is subjective. Pathologists can only assign stenosis with accuracy when it is under 30% or if it is over 70%.[6,7] Womack[7] demonstrated that pathologists classify stenosis as severe when the actual percentages ranged between 40% and 90% (average 67%). This may be attributed to various examiner biases. In order to examine the potential observer biases inherent in macroscopic visual assessment a reliable objective method of assessing coronary artery stenosis is required. A study focussing solely on the value of the histological assessment of coronary disease autopsy has not been found in the literature.

With the macroscopic method, the pathologist has to visually correct for the different types of coronary narrowing (concentric or eccentric) as well as the lack of pressure in post-mortem arteries.[8,9] Diagrammatic schemes have been developed to indicate the

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appearance of different levels of stenosis with each type of narrowing.[10] However, it is open to observer biases. This problem can be by-passed with the histological method by using formalin fixed tissue at transmural pressure which roughly recreates the artery in its *in vivo* shape.[11]

In addition, pathologists may be unintentionally swayed due to their education and training or by existing trends in death certification.[12–14] Is coronary disease the "default" for cause of death?[13] An attempt to address this question was undertaken using data from the Framingham Heart Study.[15] They concluded that coronary disease is overestimated especially in the elderly.

Histological examination can be performed by visual assessment or by using image analysis techniques.[16] Ford et al.[16] demonstrated that visual histological assessment is more objective and has very good reliability rates compared to gross morphological examination. It was also shown that previous experience does not affect observer reproducibility, but training does decrease the number of outliers. The study proposed here uses image analysis which does not allow for visual error and is presumed to be even more objective than the visual histological assessment used by Ford et al.[16]

Although this method is precise, is it accurate? Ford et al.[17] performed a follow-up study where it was found that in general there was an underestimation of stenosis. The underestimation was attributed to tissue shrinkage, whereby a decrease in the area of tissue occurs without a decrease in the luminal area. It could also be due to the fact that the more experienced observers tended to estimate stenosis in mid-ranges and away from higher levels of stenosis. Isner et al.[18] showed that using visual microscope analysis yielded an 8% increase in the number of cases being classified as severe when compared to image analysis.[18] This demonstrates the possibility of intra-observer bias when not using image analysis.

Another source of potential error could be arterial 'remodelling'. This is a mechanism of compensatory dilation of up to 40%–60% arterial expansion. It occurs alongside atherosclerotic plaque development for the purpose of luminal patency and involves tunica media smooth muscle cell reorganisation.[6,17] The process of remodelling seems to depend on the features of the plaque itself and its rate of growth; and not on the individual in question.[6] Stenosis estimation adheres to the idea that intimal thickening will occur inwards, however, during remodelling this may occur as an outward expansion of intimal thickness to avoid compromising the haemodynamic status of the artery.[17]

The two methods of assessing coronary stenosis are well known but only macroscopic investigation takes place at all autopsies. The prevalence of microscopic examination depends on the guidelines of each forensic department and country. Two reports: one from the council of Europe and one from the United States state different recommendations on the routine use of histology as a method [19,20] The Royal College of Pathologists in Great Britain advises histological sampling of major organs as part of its instructions.[21] The forensic autopsy protocol in France does not always include a histological examination.[22] In Germany, the histological examination must be ordered by the Prosecutor Fiscal. All natural deaths are subject to a visual examination and even though histological samples are taken and stored they are not routinely examined. This has been mainly attributed to the high cost of the additional examination (E. Kranioti, personal communication, 29 Mar 2011). On the contrary, in Greece, the protocols vary among forensic departments.[23] In Crete, for instance, standard procedure demands sampling and histological examination of all organs, including coronary arteries in all cases. Due to the lack of standardisation in the guidelines on the routine use of histology it is plausible that depending on the institution that pathologist was trained in and where they continue to work will influence their decisions on method choice. When considering the admissibility of evidence in court the pathologist should not rely on visual evidence due to its demonstrated subjectivity.[24] Histological analysis may be indispensable to refine, contest or verify macroscopic findings in forensic cases.[22]

This study aims to compare macroscopic and quantitative microscopic image analysis in the assessment of stenosis in patients with coronary atherosclerosis. The hypothesis to be tested is whether the quantification of stenosis using modern image techniques and cross-section histology would be a useful complementary tool for the examination of the coronary vessels and the accurate establishment of cause of death in cases of sudden cardiac death in a forensic setting.

2. Methods

Coronary arteries from 131 individuals, all of whom had died of natural causes, were included in this study. 114 originated from the Department of Forensic Sciences, Medical Faculty, University of Crete, Crete, Greece and 17 were from the archives of the Medical Examiner's Office, Oakland County, Michigan, USA. There were two groups: a control group ($n = 9$) and an atherosclerotic group ($n = 122$). In the atherosclerotic group there were 105 males and 17 females with an age range of 23–98 years (average 57.7 years).

Standard coronary stenosis assessment was performed by the pathologist during autopsy.[1] The degree of stenosis was assessed macroscopically by the pathologist. The major epicardial arteries were then removed (the left anterior descending branch, left circumflex and right coronary artery). The most severely occluded artery was processed for histological analysis. The samples were perfusion-fixed at transmural pressure in 10% formaldehyde for 24 h at room temperature. They were paraffin embedded and stained with H&E or a Trichrome stain. Blind analysis was performed on Image-Pro Plus™ 6.0 (Media Cybernetics, Maryland USA, 2006). Each measurement was performed twice for comparison. Statistical analysis was performed using an independent samples *T*-test using Minitab™ 15 software (Minitab Inc., USA). Statistical significance was set at $p < 0.05$.

3. Results

3.1. Significant atherosclerosis (>75%) and cause of death

The control group ($n = 9$) had non-significant stenosis in all cases and the same result was achieved using both methods. The atherosclerotic group ($n = 122$), however, revealed a difference between the two methods. The macroscopic examination gave non-significant stenosis in 1 individual that according to microscopic analysis had significant stenosis. The microscopic examination showed significant stenosis in 108 individuals and non-significant stenosis in 14 individuals. For each of the 14 individuals the macroscopic estimation yielded a diagnosis of significant stenosis. This corresponds to a decrease of significant stenosis in 11.5% ($n = 14$) of cases from the atherosclerotic group. In these 14 cases the cause of death was given as coronary disease.

3.2. Correlation between microscopic and macroscopic assessment

3.2.1. Overall

The results of the methods were statistically analysed in 111 cases. For the other 11 cases gross estimation was not given as an exact percentage only as >75%. In these individuals the histological estimation ranged from 67% to 94%. The average microscopic estimation was 86.0% and the average macroscopic estimation was 89.5%. This corresponds to the macroscopic estimation being on

Table 1
Microscopic estimation.

Microscopic estimation	*N*	Mean% Micro	Mean% Macro	Mean% Difference[a]	Standard error	*T*-value
<75%	13[c]	71.1	87.3	−16.2	2.2	[b]7.2
75%–80%	17	78.1	89.4	−11.3	1.9	[b]5.8
81%–85%	25	82.9	88.4	−5.5	1.6	[b]3.3
86%–90%	18	88.1	91.1	−3.0	1.5	1.9
91%–95%	19	93.2	88.8	4.4	1.9	[b]2.3
96%–100%	19	98.0	91.7	6.2	1.6	[b]3.9

[a] Negative values indicate that the macroscopic estimation was greater and positive values indicate that the microscopic estimation was greater.
[b] *T*-differences between the microscopic results at different levels of stenosis with the corresponding macroscopic result ($n = 111$). $p < 0.05$.
[c] $n = 13$ because one was excluded from statistical analysis as the macroscopic estimate was given as >75%.
Note for both tables that the overall numbers for some of the groups may appear similar. This does not infer that each individual was assigned to the same percentage range by both methods of assessment.

average, 3.5% greater than the microscopic estimation. The two methods were significantly different ($p = 0.001$; *T*-value = 3.2; standard error = 0.8).

3.2.2. Microscopic quantitative image analysis

The microscopic method was analysed separately in six groups and correlated with its corresponding value from the macroscopic method (Table 1). Statistically significant differences occurred from the ranges from <75% to 85% and from 91% to 100%. At lower percentages of microscopic stenosis the difference between the two methods was at its greatest. The macroscopic estimation was larger than the microscopic estimation until the estimation reached 91% and above.

3.2.3. Macroscopic examination

The macroscopic method was compared to the microscopic method at seven different ranges of stenosis (Table 2). Statistically significant differences occurred from the ranges 75% to 80% and above 86%. At higher percentages of macroscopic stenosis, the difference between the two methods was greater than at lower levels. The macroscopic estimation was larger than the microscopic estimation in the last four groups (81%–100%).

3.3. Intra-observer error of the quantitative image analysis method

The intra-observer rate of the microscopic method was assessed and statistically analysed. There was a mean difference of 0.1% between the assessments ($p = 0.96$; $t = 0.05$; standard error difference = 1.9).

Table 2
Macroscopic estimation.

Macroscopic estimation	*N*	Mean% Micro	Mean% Macro	Mean% Difference[a]	Standard error	*T*-value
<75%	1	82.0	65.0	17.0	n/a	n/a
75%	7	85.4	75.0	10.4	4.1	[b]2.6
80%	15	86.4	80.0	6.4	1.9	[b]3.4
81%–85%	14	81.0	85.0	−4.0	2.4	1.7
86%–90%	30	86.4	90.0	−3.6	1.4	[b]2.6
91%–95%	30	87.7	95.2	−7.5	1.7	[b]4.4
96%–100%	14	86.7	100.0	−13.3	2.5	[b]5.4

[a] Negative values indicate that the macroscopic estimation was greater and positive values indicate that the microscopic estimation was greater.
[b] *T*-differences between the macroscopic results at different levels of stenosis with the corresponding microscopic result ($n = 111$). $p < 0.05$.
Note for both tables that the overall numbers for some of the groups may appear similar. This does not infer that each individual was assigned to the same percentage range by both methods of assessment.

4. Discussion

Coronary artery stenosis is considered significant if it reaches levels of 75% in at least one of the main coronary arteries.[1] Often there is no documented medical history and macroscopic autopsy findings may be lacking. This necessitates the need for ancillary methods to be used such as histological analysis.[25] This study focuses on the quantification of stenosis using quantitative histological image analysis. The method was compared to visual gross estimation of stenosis and the results indicated that the two methods were significantly different from each other ($p = 0.001$).

In a total of 122 patients diagnosed with coronary disease during autopsy, the macroscopic method gave a greater percentage stenosis by an average of 3.5%. In 14 cases, (11.5%) macroscopic diagnoses produced a classification of significant atherosclerosis (>75%) while the microscopic estimation yielded a non-significant level of stenosis. The results were broken down by method into ranges and correlated with their corresponding value from the other method. In general, at lower percentages of microscopic assessment there was a lower correlation between the methods (Table 1). Conversely, at higher levels of macroscopic assessment there generally was a decrease in the level of correlation between the two methods (Table 2). Methodological issues and examiner biases are the two most likely reasons to cause the discrepancies between the methods. The gross evaluation in this study gave on average a 3.5% greater estimation of stenosis. This could be due to one of three reasons: the microscopic method underestimates stenosis; the macroscopic method overestimates stenosis; or both these errors occur simultaneously.

Histological underestimation may be due to tissue shrinkage which can distort the assessment of stenosis as it decreases the area of the arterial wall without reducing the luminal area.[26] The formaldehyde fixative used in this study causes minimal shrinkage, however, subsequent tissue processing stages can also result in shrinkage.[27] Studies performed on quantifying aspects of arteries give different percentages of shrinkage, ranging from 5% to 33%.[28] Shrinkage of 33% is thought to be extreme. In the cases where significant stenosis was disputed the average discrepancy was 16.2%. Shrinkage would have to be above this level to completely account for these differences between the two methods. As this was a retrospective study it is impossible to give actual percentage shrinkage and this methodological artefact may therefore contribute to the differences.

If enlargement of the atherosclerotic plaque occurs with subsequent or simultaneous remodelling it can yield an overestimation in stenosis. [30,31] This is especially relevant when considering image analysis as it does not allow for any extra information to be computed when estimating stenosis. Also, this compensatory mechanism can allow for up to 90% luminal stenosis before a fatal episode. Thusly, it must be considered when deciding on cause of death. Baroldi et al.[32] assessed arterial plaque length by examining the arteries in cross section at 3 mm intervals. Lumen stenosis was calculated as a percentage of the normal diameter measured in areas of coronary arteries where no stenosis had occurred using plastic cast moulds. This method of assessment factors the presence of compensatory dilation before giving an estimation of stenosis. However, in this particular study the image analysis may have underestimated and not overestimated when compared to the equivalent gross visual assessment. Ford et al.[17] discussed tissue shrinkage underestimation coinciding with the possibility of overestimation of stenosis as a result of remodelling. They theorised that this may compensate somewhat for the

possible tissue shrinkage artefact and bring the estimation closer to *in vivo* state.[17]

The macroscopic method is a subjective visual method. There are many sources of bias that could cause the overestimation and a general decrease in correlation between the methods. The lumen shape and lack of *in vivo* pressure needs to be acknowledged.[8,17] Fixing the arteries at transmural pressure subverted this issue in the histological method. This infers that any inaccuracies due to lumen shape were probably the result of the macroscopic analysis. It is worth noting that the least correlation between the macroscopic method and its corresponding microscopic percentage was when the macroscopic estimation was at 100%. This could indicate that the shape may have played a role in the discrepancy. The lack of *in vivo* pressure may cause the lumen to collapse. Consequently the pathologist may have deemed it to be fully occluded when it in fact it was only somewhat collapsed. This may account for the decrease in correlation at higher levels of macroscopic assessment. Conversely, at lower levels of microscopic estimation there was a decrease in correlation. This may be due to the fact that gross examination is only able to accurately assess stenosis when it is less than 30% or more than 70%.[7,16,29] The findings of this study support this since when the microscopic assessment was $<75\%$ there was the least correlation between the two methods.

Other biases for the macroscopic method include examiner's having a different training background or current trends in medical practice that may subconsciously alter the pathologist's decisions. In this study the method using quantitative image analysis does not allow for any extra information to be inadvertently included by the examiner and this reduces observer error rates. The intra-observer error rate for this method is very good and on average the difference in the two measurements taken was 0.1%. Overall, observer error rates are more likely to be higher when using macroscopic analysis. This is due to its subjective visual nature, as well as the different experience and training of each pathologist.

An accepted, consistent diagnostic examination of the heart is needed across all pathology services but in particular within the forensic autopsy. Ultimately, a valid and verifiable recording of the cause of death is a vital requirement for quality assurance. This issue could be addressed with peer review, standardised protocols and adequate sampling for audit. This is not always feasible due to money and time constrains placed on forensic institutes.[33] Using retrospective studies (such as this one) may utilise information already available to pathology services without extra histological costs. If all histological specimen already archived were compared with autopsy report for degree of stenosis a much larger sample size would be immediately attainable. It would also provide a cross reference for different pathologists grossly assessing stenosis. It therefore might be able to highlight certain biases in estimation as discussed above. Once data is fully documented it can provide a great archive that will be able to help diagnostic research and public health trends. Moreover, in cases of forensic autopsies it is paramount to have reliable and verifiable data due to the possibility of further legal proceedings.[4,22,24] Quantitative histological image analysis may subvert biases and provides accurate percentages (once tissue shrinkage is taken into account). In this study it is thought that a level of 33% is extreme and that the level of shrinkage should be much lower. Quantifying the degree of tissue shrinkage must be done before tissue processing is complete. It is an essential component of research for future standardisation of forensic autopsy of the heart. The macroscopic method is open to many examiner biases and may be too subjective for the establishment of cause of death in cases of sudden cardiac death in forensic settings. In order to achieve an exact percentage of stenosis and to put this information in the context of the coronary vessels and of the autopsy as a whole it may be necessary to use the macroscopic method in conjunction with modern image techniques and cross-section histology.

Conflict of interest

None declared.

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Ethical approval

None.

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